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10/518,749	12/22/2004	Takashi Nakayama	1422-0651PUS1	3018

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EXAMINER	
SGAGIAS, MAGDALENE K	

ART UNIT	PAPER NUMBER
1632	

NOTIFICATION DATE	DELIVERY MODE
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

mailroom@bskb.com

Office Action Summary	Application No.	Applicant(s)	
	10/518,749	NAKAYAMA ET AL.	
	Examiner	Art Unit	
	MAGDALENE K. SGAGIAS	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 December 2007*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-18* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-18* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's arguments filed 12/20/07 have been fully considered but they are not persuasive. The amendment has been entered. Claims 1-18 are pending and under consideration. Claim 19 has been canceled.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims **1-8, 10-18** are rejected under 35 U.S.C. 102(b) as being anticipated by **Weiss et al,** (US 5,981,165).

Weiss et al, teaches a method for producing isolated neural cells, by culturing a suspension of embryonic stem cells in the presence of ingredients equivalent to an astrocyte conditioned medium, (column 12, example 3) **(claims, 1, 10-11, 13, 16-18)**. Further Weiss teaches the formation of neurospheres (figure 2) (**claims 4-8**).

Weiss teaches the embryonic stem cells are murine (**claims, 2-3**).

Weiss teaches a method of producing a neuron by carrying a suspension of embryonic stem cells in the presence of ingredients substantially equivalent to an astrocyte conditioned medium in the state of adhesion of the neural stem cells to an adhesive culture substratum by plating the cells onto poly-L-ornithine coated glass cover slips, in the complete medium with rat B49 glial cell line-derived conditioned medium in the absence of bFGF, in the presence of FGF2

and in the presence of ingredients substantially equivalent to astrocyte conditioned medium (example 8) (**claim, 10**).

Weiss teaches the isolated neuron expresses tyrosine hydroxylase (example 2) (**claim 14**).

Thus, Weiss anticipates the claimed invention.

Applicants argue that the "embryonic stem cells" disclosed in Weiss are neural stem cells isolated from the brains of El4 (embryonic day 14) embryonic mice, (i.e., adult (somatic) stem cells), and such neural stem cells are clearly different from the ES cells (embryonic stem cells) as taught in the present invention, which are pluripotent cells established from blastocysts (E3.5 blastocysts).

These arguments are not persuasive because the invention as claimed does not require pluripotent cells established from E3.5 blastocysts.

Applicants argue there is no evidence that the cells isolated in Weiss are neural stem cells (differentiation into neurons, astrocytes, or oligodendrocytes). Moreover, it is likely that the isolated cells are neural precursor cells committed to the neurons. The astrocyte conditioned medium (ACM) contains a neurotrophic factor for the neural precursor cells once committed to the neurons, and allows the neural precursor cells to differentiate into neural cells as long as the cells are alive.

These arguments are not persuasive because the invention as claimed requires the isolated neuron to express tyrosine hydroxylase (claim 14) and Weiss et al teach the isolated neuron expresses tyrosine hydroxylase as in the claimed invention. In addition, Weiss teaches the cells are isolated in a suspension of embryonic stem cells in the presence of ingredients substantially equivalent to an astrocyte conditioned medium in the state of adhesion of the

neural stem cells to an adhesive culture substratum by plating the cells onto poly-L-ornithine coated glass cover slips, in the complete medium with rat B49 glial cell line-derived conditioned medium in the absence of bFGF, in the presence of FGF2 and in the presence of ingredients substantially equivalent to astrocyte conditioned medium as in the claimed invention.

Applicants argue Weiss fails to teach each and every element of the present invention because the differentiation of the cells obtained in Weiss into neurons in the ACM is completely distinguishable from the differentiation of the ES cells or the undifferentiated neural stem cells in the ACM into neural stem cells and neural cells as taught in the present invention.

These arguments are not persuasive because applicants have not provided evidence or arguments as to how the cells obtained by Weiss are distinguishable from the cells claimed in the present invention. Weiss et al teach all the elements of the claimed invention that is isolated neuron to express tyrosine hydroxylase and the isolated neuron expresses tyrosine hydroxylase as in the claimed invention. In addition, Weiss teaches the cells are isolated in a suspension of embryonic stem cells in the presence of ingredients substantially equivalent to an astrocyte conditioned medium in the state of adhesion of the neural stem cells to an adhesive culture substratum by plating the cells onto poly-L-ornithine coated glass cover slips, in the complete medium with rat B49 glial cell line-derived conditioned medium in the absence of bFGF, in the presence of FGF2 and in the presence of ingredients substantially equivalent to astrocyte conditioned medium as in the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims **1-3, 10-12** are rejected under 35 U.S.C. 103(a) as being unpatentable over **Zhang et al,** (Nature Biotechnology, 19: 1129-1133, 2001, IDS) in view of **Flax et al,** (Nature, 16: 1033-1039, 1998).

Zhang teaches human embryonic stem (ES) cell-derived neural precursors generate all three CNS cell types in vitro and the isolated neural precursors expanded as free-floating cell aggregates in a suspension similar to "neurosphere" cultures (p 1129, 2nd column last paragraph). Zhang et al teach upon aggregation of embryoid bodies (EBs), differentiating ES cells formed large numbers of neural tube-like structures in the presence of FGF-2, wherein neural precursors within these formations were isolated and purified on the basis of differential adhesion (abstract). Zhang et al teach following withdrawal of FGF-2 they differentiated into neurons, astrocytes and oligodendrocytes (abstract). Zhang teaches the in vitro differentiation of the ES cell-derived neural precursors was induced by withdrawn of FGF-2 and plating on the state of adhesion of the neural stem cell precursor by plating on ornithine and laminin substrate (p 1130, 1st column, 2nd paragraph). After 7-10 days after plating differentiated neurons expressed neuronal markers MAP2ab, β_{II}-tubulin, GABA, tyrosine hydroxylase (TH), GFAP (p 1130, 2nd column, 1st paragraph). Zhang teaches the suspension of human ES in ingredients substantially equivalent to an astrocyte conditioned medium and the absence of EGF (p 1132-1133 and figure 3). Zhang teaches on a pragmatic level, the in vitro generation of neural tube-like structures and the possibility of isolating these structures on the basis of their differential adhesion provides a simple yet efficient approach for generating human ES-derived neural precursors in high purity (p 1131, 2nd column, 3rd paragraph). Zhang suggests because

undifferentiated ES cells and precursors to other lineages may form tumors and foreign tissues, the generation of purified somatic populations of cells is a key prerequisite for the development of ES cell-based neural transplant strategies (p 1131, 2nd column, 3rd paragraph). Zhang teaches the chemically defined culture system they described provides an opportunity to explore the effects of single factors on human neuroepithelial proliferation and specification in vitro (p 1131, 2nd column, last paragraph). Zhang differs from the claimed invention by not teaching the cryopreservation of neural stem cells.

However, at the time the claimed invention was made, **Flax** et al, teach functional cryopreservable human neural stem cells can be propagated in culture in vitro (p 1037-1038). As such, Flax et al provide sufficient motivation for one of ordinary skill in the art to apply the cryopreservation methodology of Flax to the neural stem cell methodology of Zhang to explore the effects of single factors on human neuroepithelial proliferation and specification in vitro.

Accordingly, in view of the teachings of Flax et al, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the neural stem cell methodology of Zhang by cryopreserving the produced neurons with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to make such a modification as Zhang has suggested the generation of purified somatic populations of cells is a key prerequisite for the development of ES cell-based neural transplant strategies.

Thus, the claimed invention as a whole, is clearly prima facie obvious in the absence of evidence to the contrary.

Applicants argue the Zhang et al. method merely performs a procedure of selecting from other cells neural precursor cells that are differentiated from ES cells at a given frequency, not a method of directing the differentiation of the ES cells directly.

These arguments are not persuasive because it is not clear as to what conditions taught by Zhang result in not a method for directing the differentiation of the ES cells directly.

Applicants argue according to the neurosphere method of Zhang, not all of the neural precursor cells can be maintained in an undifferentiated state. In addition, the selected neural cells are a mixture of three kinds, i.e., neurons, astrocytes, and oligodendrocytes that appear at the same time. On the other hand, the method of the present invention is intended to produce neural stem cells by directly differentiating the ES cells, to thereby positively direct the differentiation of the ES ceils. In addition, according to the method of the present invention, the neural stem cells derived from the ES cells can be selectively differentiated into only neurons or astrocytes. It is clear from the present specification that the neural cells are directly differentiated from embryonic stem cells. See the present specification, for example, at page 15 line 12 to page 16, line 6 and Examples 1 to 3.

These arguments are not persuasive because the claimed invention is not limited to the production of astrocytes only, even though the intention of the applicants is to produce astrocytes only.

Claim Rejections - 35 USC § 102/103

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-18 are rejected under 35 U.S.C. 102(b) as anticipated by **Zhang et al,** (Nature Biotechnology, 19: 1129-1133, 2001, IDS) or, in the alternative, under 35 U.S.C. 103(a) as obvious over **Pataky et al,** (Exp Neurol, 163(2): 357-372, 2000 (IDS)).

Zhang teaches human embryonic stem (ES) cell-derived neural precursors generate all three CNS cell types in vitro and the isolated neural precursors expanded as free-floating cell aggregates in a suspension similar to "neurosphere" cultures (p 1129, 2nd column last paragraph). Zhang et al teach upon aggregation of embryoid bodies (EBs), differentiating ES cells formed large numbers of neural tube-like structures in the presence of FGF-2, wherein neural precursors within these formations were isolated and purified on the basis of differential adhesion (abstract). Zhang et al teach following withdrawal of FGF-2 they differentiated into neurons, astrocytes and oligodendrocytes (abstract). Zhang teaches the in vitro differentiation of the ES cell-derived neural precursors was induced by withdrawn of FGF-2 and plating on the state of adhesion of the neural stem cell precursor by plating on ornithine and laminin substrate (p 1130, 1st column, 2nd paragraph). After 7-10 days after plating differentiated neurons expressed neuronal markers MAP2ab, β_{II}-tubulin, GABA, tyrosine hydroxylase (TH), GFAP (p 1130, 2nd column, 1st paragraph). Zhang teaches the suspension of human ES in ingredients substantially equivalent to an astrocyte conditioned medium and the absence of EGF (p 1132-1133 and figure 3). Zhang teaches on a pragmatic level, the in vitro generation of neural tube-like structures and the possibility of isolating these structures on the basis of their differential adhesion provides a simple yet efficient approach for generating human ES-derived neural precursors in high purity (p 1131, 2nd column, 3rd paragraph). Zhang suggests because undifferentiated ES cells and precursors to other lineages may form tumors and foreign tissues, the generation of purified somatic populations of cells is a key prerequisite for the development of ES cell-based neural transplant strategies (p 1131, 2nd column, 3rd paragraph). Zhang

teaches the chemically defined culture system they described provides an opportunity to explore the effects of single factors on human neuroepithelial proliferation and specification in vitro (p 1131, 2nd column, last paragraph). Zhang differs from the claimed invention by not teaching the culturing of stem cell spheres in the presence and then in the absence of bFGF and/or EGF for obtaining glial cell as a cell migrating from the stem cell sphere.

However, at the time the claimed invention was made, Pataky teaches that fibroblast growth factor produced differential effects on survival and neurite outgrowth from identical bulbospinal neurons in vitro. Pataky teaches that astrocytes synthesize a variety of trophic factors and astrocyte conditioned medium also promoted the survival of bulbospinal neurons (abstract).

Accordingly, in view of the teachings of Pataky et al, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the neural stem cell methodology of Zhang by progressive steps of adding bFGF or EGF to obtain neural stem cells and then culture the stem cells in the presence of bFGF or EGF to obtain glial cells with a reasonable expectation of success because Pataky states that astrocytes produce neural nutritional factors such as FGF2. One of ordinary skill in the art would have been sufficiently motivated to make such a modification as Zhang has suggested the generation of purified somatic populations of cells is a key prerequisite for the development of ES cell-based neural transplant strategies. Thus, the claimed invention as a whole, is clearly prima facie obvious in the absence of evidence to the contrary.

Applicants argue that Pataky et al fail to cure the deficiencies of Zhang et al. Pataky et al. conclude that while FGF-2 and ACM show actions of promoting for survival of bulbospinal neurons, the ACM-promoted activity is due to a factor other than FGF-2. However, Applicants respectfully submit that the Examiner has mischaracterized the Pataky et al. disclosure by

alleging that the ACM-promoted activity is due to FGF-2. According to Pataky et al., in order to identify bulbospinal neurons, a crystal of DiI is contacted with the cervical spinal cord of ES chick embryo. Three days later on E8, the brain stems are subjected to dispersion culture, and the DiI-labeled neural ceils are then identified as bulbospinal neurons, to fred the effects of FGF-2 and ACM. The principle of labeling the neural cells with a fat-soluble fluorescent dye DiI is based on diffusion within a biomembrane, which is retrograde tracing in the spinally projecting axon. In other words, cells capable of being labeled with DiI are only bulbospinal neurons of which the axon extends into the cervical spinal cord. More specifically, the effects of ACM are examined on DiI-labeled premature neural cells, which are already differentiated to extend neurites. Therefore, Pataky et al. clarify the promoting action for survival of ACM of the neurons themselves, so that Pataky et al. is completely irrelevant to the method of the present invention, which is differentiation of the ES ceils and the ES cells-derived neural stem cells in the presence of ACM.

These arguments are not convincing because Pataky et al teach **FGF-2 but not FGF-1** promoted the survival of bulbospinal neurons (see abstract and whole document). Pataky et al also teach astrocytes synthesize a variety of trophic factors, and astrocyte conditioned medium promoted the survival of bulbospinal neurons (abstract). Pataky et al suggest that neuronal cells synthesize other factors in response to exogenous FGF-2 which promote the survival of bulbospinal neurons and FGF1 promoted neurite outgrowth but not survival while the converse was true for FGF-9 and thus there are differential effects of specific growth factors on survival or neurite outgrowth of bulbospinal neurons (abstract). It is clear from the teachings of Pataky et al that astrocyte medium has the effect on neural stem cells of promoting nerve cell differentiation and in particular that astrocytes produce trophic factors such as FGF-2. Therefore, it is clear from the teachings of Pataky et al that astrocyte medium contains factors that promote nerve

cell differentiation and as such, Pataky et al provide sufficient motivation for one of ordinary skill in the art to use astrocyte medium in addition to the FGF-2 used by Zhang et al to promote differentiation of ES cells into glial cells into the culture conditions of Zhang et al.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims **1, 4, 7-8, 10, 11, 13-18** rejection under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is withdrawn.

Conclusion

No claim is allowed.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Magdalene K. Sgagias whose telephone number is (571) 272-3305. The examiner can normally be reached on Monday through Friday from 9:00 am to 5:00 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras, Jr., can be reached on (571) 272-4517. The fax phone number for the organization where this application or proceeding is assigned is (703) 872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll free).

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